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Structural analysis of the female reptile reproductive system by micro-computed tomography and optical coherence tomography†

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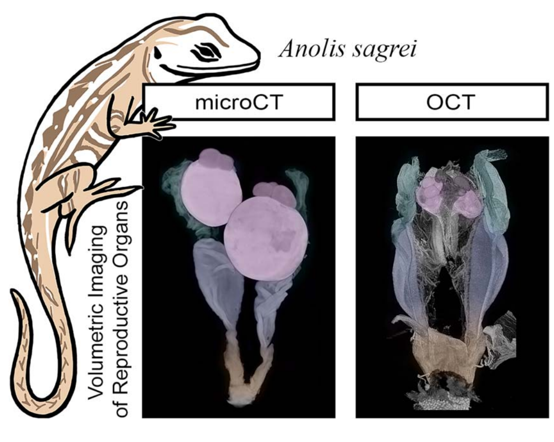
Abstract

Volumetric data provide unprecedented structural insight to the reproductive tract and add vital anatomical context to the relationships between organs. The morphology of the female reproductive tract in non-avian reptiles varies between species, corresponding to a broad range of reproductive modes and providing valuable insight to comparative investigations of reproductive anatomy. However, reproductive studies in reptilian models, such as the brown anole studied here, have historically relied on histological methods to understand the anatomy. While these methods are highly effective for characterizing the cell types present in each organ, histological methods lose the 3D relationships between images and leave the architecture of the organ system poorly understood. We present the first comprehensive volumetric analyses of the female brown anole reproductive tract using two non-invasive, non-destructive imaging modalities: micro-computed tomography (microCT) and optical coherence tomography (OCT). Both are specialized imaging technologies that facilitate high-throughput imaging and preserve three-dimensional information. This study represents the first time that microCT has been used to study all reproductive organs in this species and the very first time that OCT has been applied to this species. We show how the non-destructive volumetric imaging provided by each modality reveals anatomical context including orientation and relationships between reproductive organs of the anole lizard. In addition to broad patterns of morphology, both imaging modalities provide the high resolution necessary to capture details and key anatomical features of each organ. We demonstrate that classic histological features can be appreciated within whole-organ architecture in volumetric imaging using microCT and OCT, providing the complementary information necessary to understand the relationships between tissues and organs in the reproductive system. This side-by-side imaging analysis using microCT and OCT allows us to evaluate the specific advantages and limitations of these two methods for the female reptile reproductive system.

Summary Sentence

Two volumetric imaging modalities, contrast-enhanced micro-computed tomography and optical coherence tomography, facilitate visualization of the macro- and micro- architecture of the reproductive tract of the female brown anole, *Anolis sagrei*.

Graphical Abstract



Key words: DICECT, OCT, volumetric, imaging

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Introduction

The morphology of the non-avian reptilian female reproductive tract varies between species. While the generalized architecture of this system is relatively consistent across this diverse group of animals, species-specific modifications have evolved, corresponding to a broad range of reproduction modes. While many species are egg-laying (oviparous), many reptile species are viviparous, giving birth to live young. In reptiles, viviparity has evolved over 100 times [1], including 6 origins of derived matrotrophy, which is the direct maternal delivery of nutrients to developing embryos [1].

The reproductive organ system in most female reptiles consists of left and right ovaries that are located medially, containing follicles that increase in size with oocyte maturation, along with left and right reproductive tracts, which are the site of gamete and embryo transport and development. The ovaries are located cranially to two independent ducts that connect caudally at the cloaca along with the digestive tract. The reproductive tract contains morphologically distinct regions that include, from cranial to caudal, the infundibulum, the glandular uterus, and the nonglandular uterus. Ovulated oocytes enter the infundibulum, a thin tube with both ciliated and secretory cells [2]. The most proximal infundibulum is funnel-shaped and has a highly ciliated lip [3]. The glandular uterus is the location where a fertilized oocyte will reside for ∼18 days of embryonic development in anoles [4]. In oviparous species, the glandular uterus is the site where the albumin and eggshell are deposited. The nonglandular uterus consists of a cervix and vagina, connecting the glandular uterus to the cloaca. The nonglandular uterus is a highly muscular tube whose tissue architecture transitions from cranial to caudal [3].

The brown anole, which is the subject of this study, is a popular study organism and is part of a well-studied genus of over 400 species [5]. This species lays approximately 1 egg per week during the summer breeding season [6, 7] and females are capable of sperm storage [8]. As with other reptiles, the brown anole has two independent reproductive tracts comprised of an infundibulum, glandular uterus, and nonglandular uterus. Historical investigation of the *Anolis* reproductive system has been limited to histological studies [9–16]. There is a good understanding of the cell types of the reproductive tract organs [2, 8]. However, the architecture of the reproductive tract in this species is relatively poorly understood [3, 14] due to the two-dimensional (2D) nature of histological studies.

Recently, micro-computed tomography (microCT) has been used to generate volumetric structural data and gain a more comprehensive understanding of the macro-architecture of the nonglandular uterus in the female reproductive tract [3]. MicroCT is a high-resolution, non-destructive, volumetric imaging method that has been applied to diverse fields of study. MicroCT has been applied to studies of reproductive biology across species including *Drosophila* [17], millipedes [18], reptiles [19–21], and mammals [22–25]. MicroCT reconstructs volumetric structural information using a series of two-dimensional projection X-radiographs acquired at different orientations of the object. While this methodology has been traditionally used to image dense materials like bone, soft tissues can be visualized using radio-opaque contrasting agents. Though many contrasting agents can be used, Lugol's iodine is a popular choice for microCT in a method referred to as diffusible iodine-based contrast-enhanced computed tomography or DICECT [26]. Recently, we used contrast-enhanced microCT, specifically DICECT, to understand the 3D architecture of the nonglandular uterus of the brown anole lizard. Our data allowed us to interpret historical histological studies to facilitate species anatomical comparisons [3].

While microCT provides detailed structural information of samples, and particularly reptile reproductive organs, it is associated with some limitations. Tissue fixation, staining, and processing for microCT is time-consuming; taking multiple days, this preparation might result in structural disturbances due to dehydration and is not compatible with live imaging. Another imaging technology, optical coherence tomography (OCT), has the potential to address these limitations and provide complementary information. OCT is an up-and-coming imaging modality in the fields of development and reproduction [23, 27]. OCT is a non-invasive, label-free, and depth-resolved optical imaging modality with a micro-scale spatial resolution (1–10 μm) at an imaging depth of approximately 2–3 mm in biological tissues. OCT measures the location of light scatter within a sample by detecting the echo delay in backscattered light samples. Dynamic imaging is possible with OCT as three-dimensional data across a wide field of view can be collected at a high rate. Due to its non-destructive nature, OCT is gaining popularity in the fields of developmental [28, 29] and reproductive [23, 27] biology to investigate dynamic processes of germ cells (eggs and sperm) and embryos in live mammalian tissues. However, the application of this technology for the study of reproductive processes has not yet extended to reptiles.

In order to broaden our understanding of reptilian reproductive anatomy and set a platform for future imaging studies, we comparatively investigated the use of two complementary volumetric imaging techniques, contrast-enhanced microCT and OCT, for volumetric imaging of the female reproductive tract of the brown anole, *Anolis sagrei*.

Methods

Micro-computed tomography

The animals used for microCT analyses were collected and euthanized at the University of Florida during the summer of 2017 with approval by the University of Florida Intuitional Animal Care and Use Committee (IACUC). Lizards were euthanized with an intraperitoneal injection of Euthasol, fixed with 10% formalin, and immersed in 1.75% aqueous Lugol's iodine according to established protocols for DICECT [26]. The animals Anoles 1, 2, and 3 were scanned on a Phoenix V|Tome|X M at the University of Florida Nanoscale Research Facility. Tomograms were generated from radiographs using Datos XR. Postprocessing and segmentation were performed using 3D Slicer [30] and Imaris (Oxford Instruments Group). MicroCT scanning parameters and tomograms were uploaded to MorphoSource and are available for download (MorphoSource specimen ID: 000590186, 000608703, and 000608693).

Optical coherence tomography

The animals used for OCT analyses were collected at the Houston Arboretum during the summer of 2022 with approval by the University of Texas MD Anderson Cancer Center IACUC. These lizards were maintained in the lizard colony until March 2022, March 2023, and October 2022 when they were anesthetized by intracoelomic injection of 1% tricaine (MS-222) and euthanized with an intracoelomic injection of 50% tricaine [31]. The extracted reproductive organs were then imaged with one of two OCT systems. The first system (OCS1310V1, Thorlabs), used for Anole 4 and 5, uses a swept-source laser (SL1310V1, Thorlabs) with a central wavelength of 1300 nm and a scan range of ~100 nm. This imaging system allows for an imaging depth of up to 12 mm, an A-line rate of 100 kHz, and axial and transverse resolution of ~12 and ~16 μm, respectively. The second OCT system, used for Anole 6, is a house-built OCT system using a supercontinuum laser (NKT Photonics) with a central wavelength of ~800 nm and a bandwidth of ~100 nm in a fiber-based Michelson interferometer. The interference between light reflected by the reference and sample arms is directed to a spectrometer based on a 250 kHz e2V OctoPlus camera (Teledyne Technologies Inc.) and Fourier transformed to obtain the structural OCT intensity. To image over a volume, galvanometer mirrors (GVS012, Thorlabs Inc.) provide a high degree of flexibility in scan area and density. This system allows for a maximum A-line rate of 250 kHz and axial and transverse resolution of ~4 μm. To image over a volume, galvanometer mirrors provide a high degree of flexibility in scan area and density. Each OCT system was controlled using custom LabVIEW software (National Instruments) to acquire data with imaging settings selected to account for size and structural detail throughout the reproductive organs. Data processing was performed in Matlab (MathWorks) with custom scripts. Volumetric renderings were compiled using Imaris software (Bitplane).

Quantitative measurements

To quantify the thickness of the wall of the different regions of the reproductive tract from the volumetric OCT and microCT imaging data sets, we collected measurements from the infundibulum, the upper, middle, and lower glandular uterus, and the most caudal region of the nonglandular uterus, the vagina. At each region, we made linear measurements from the lumen to the outer wall of the reproductive tract in cross-sections oriented perpendicular to the lumen. Measurements were collected from each of six anole lizards, three imaged by microCT and three by OCT. In each animal, we collected five measurements per location. For the microCT scans, we performed these measurements in FIJI [32], and for the OCT scans, in Imaris (Oxford Instruments Group) software. We plotted the data in Python.

Results

Architecture of the female brown anole reproductive tract

The anatomy of the brown anole reproductive tract from cranial to caudal is ovary, infundibulum, glandular uterus, nonglandular uterus, and cloaca (Figure 1A). We demonstrate the organization of the reproductive tract in the brown anole lizard using brightfield microscopy, where each organ can be clearly visualized (Figure 1B). We performed the first volumetric comparison of microCT (Figure 1C, Supplementary Video S1) and OCT (Figure 1D, Supplementary Video S2) imaging of the complete reproductive tract from the brown anole lizard. The microCT data displayed is segmented from a full-body scan and the OCT is stitched from smaller scans. We demonstrate that the architecture of the reproductive tract is clearly discernible in both datasets, including the ovaries, infundibulum, glandular and nonglandular uterus.

Architecture of the female brown anole ovary

We compared microCT (Figure 2A) and OCT (Figure 2B) volumetric imaging of the ovary. In coronal, digital cross-section through comparable portions of the ovaries from each dataset (Figure 2C and D), ovarian follicles are clearly visible. We observe follicles at different stages of maturation, which increase in volume. Notably, the female lizard in the microCT dataset shown was imaged during the breeding season and exhibits a large follicle on each ovary that has accumulated a large amount of yolk and is pre-ovulatory; the lizard in the OCT dataset shown was imaged during the non-breeding season and the ovarian follicles are relatively small. In the microCT dataset where follicles of varying maturity are present, we observe density changes (in midsized follicles, the outer edge is more dense than the center) as the follicles increase in size (i.e. mature) (Figure 2C). In the relatively immature follicles present in our OCT dataset, we do not see changes in optical density (Figure 2D).

Architecture of the female brown anole infundibulum

We compared microCT (Figure 3A) and OCT (Figure 3B) volumetric imaging of the infundibulum, which lies laterally to the ovary and connects to the glandular uterus. In both datasets, we observe that the infundibulum is a highly folded tube compromised of a narrow lumen surrounded by a thin wall. In sagittal, digital cross-section through the infundibulum of the microCT dataset, the wall of the tube is visible; however, the resolution of the full-body CT scan makes the lumen less clearly distinguished (Figure 3C). The superior resolution of the OCT imaging improves the ability to distinguish the walls of the tube from the lumen (Figure 3D).

Architecture of the female brown anole glandular uterus

We compared microCT (Figure 4A) and OCT (Figure 4B) volumetric imaging of the glandular uterus. The glandular uterus is a thicker tube that connects the infundibulum to the nonglandular uterus. MicroCT (Figure 4A) and OCT (Figure 4B) both show the glandular uterus wall clearly, which is thicker and less folded than the infundibulum. In a coronal cross-section though the glandular uterus in our microCT dataset, the lumen is clearly defined and is surrounded by a thick wall (Figure 4C). Similarly, coronal OCT cross-sections demonstrate a thick tube with a well-defined lumen (Figure 4D). However, due to limitations in OCT imaging depth, it is not possible to appreciate the entirety of the tube.

Architecture of the female brown anole nonglandular uterus

We compared microCT (Figure 5A) and OCT (Figure 5E) volumetric imaging of the nonglandular uterus. The nonglandular uterus is a short, muscular tube connecting the glandular uterus to the cloaca and exhibits a highly complex luminal morphology. The defining features of this complex lumen can be clearly observed in the coronal cross-sections through both microCT (Figure 5A-D) and OCT (Figure 5E-H) datasets.

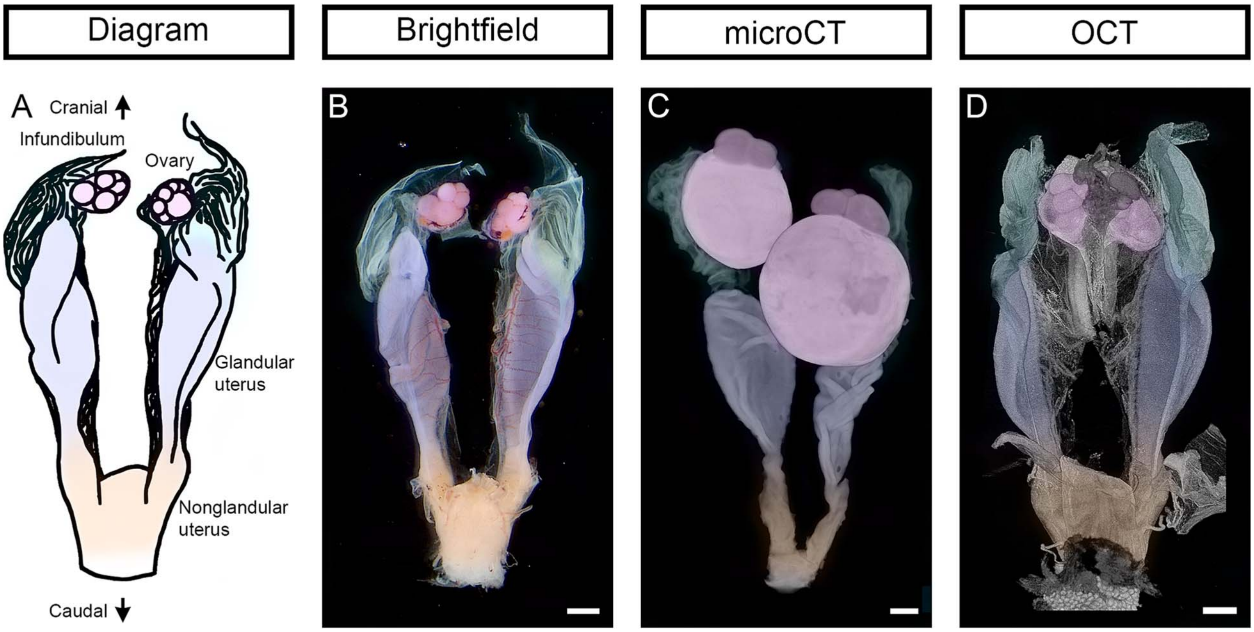

Figure 1. Overall structure of the female reproductive tract of anole lizards. (A) The diagram of the reproductive tract showing the infundibulum, ovaries, glandular, and nonglandular uterus. (B) Corresponding bright-field microscopic image of the extracted reproductive system. (C) Volumetric rendering of the reproductive system, imaged with microCT. Imaging was performed within the animal and the reproductive system was digitally segmented from the acquired data. (D) Volumetric OCT imaging of the whole extracted reproductive system. The image is compiled from three datasets acquired with an overlap and stitched together. The anatomical structures throughout the paper are color-coded: infundibulum (cyan), ovaries (magenta), glandular uterus (blue), and nonglandular uterus (orange). The scale bars correspond to 1 mm.

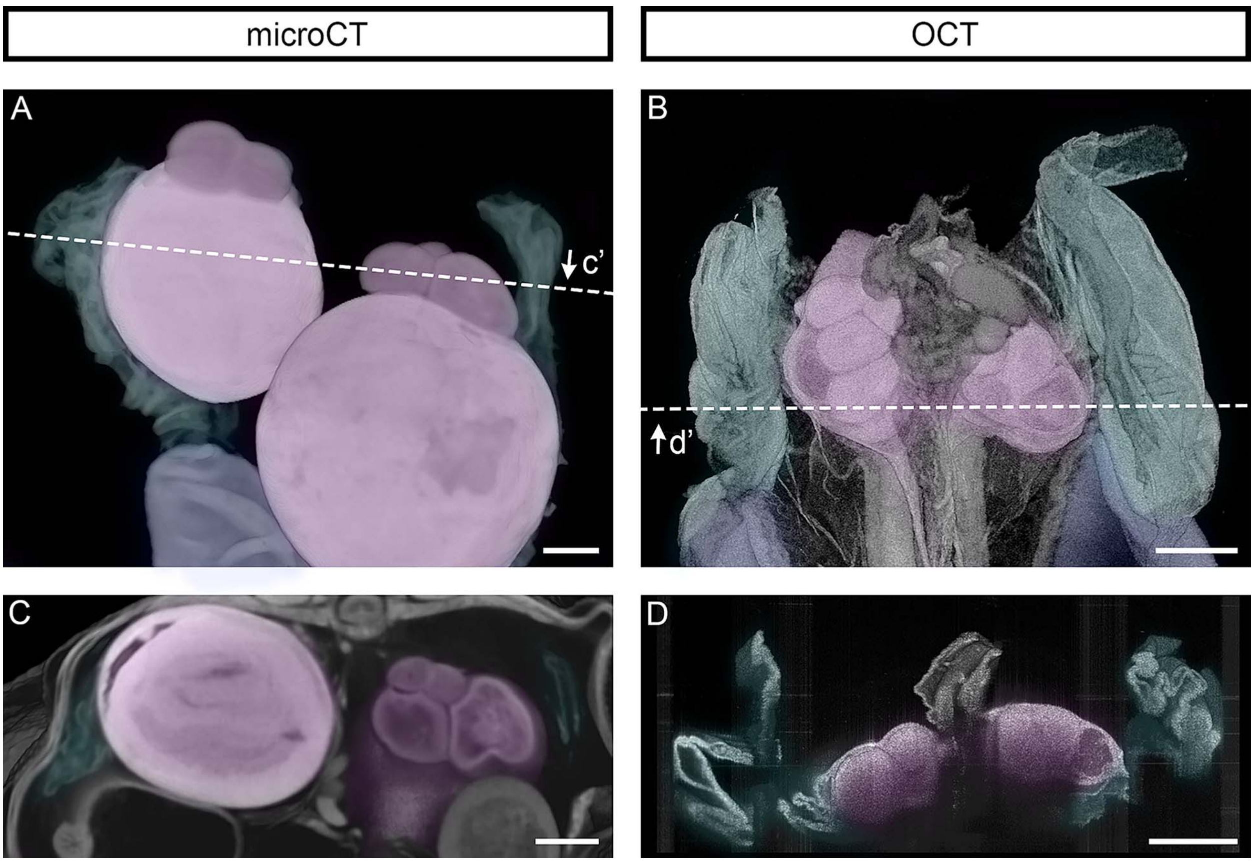

Figure 2. Volumetric imaging of anole lizard ovaries. (A) Anterior volumetric view of microCT scan showing ovaries among the digitally segmented reproductive organs. (B) Anterior view of the ovary imaged with OCT. (C) Volumetric view from the cross-sectional plane identified by the dashed line c′ in panel A showing the microCT scan from the whole body including other organs. (D) Volumetric view of OCT scan from the cross-sectional plane identified by the dashed line d′ in panel B. Anatomical structures are color-coded: infundibulum (cyan), ovaries (magenta), and glandular uterus (blue). The scale bars correspond to 1 mm.

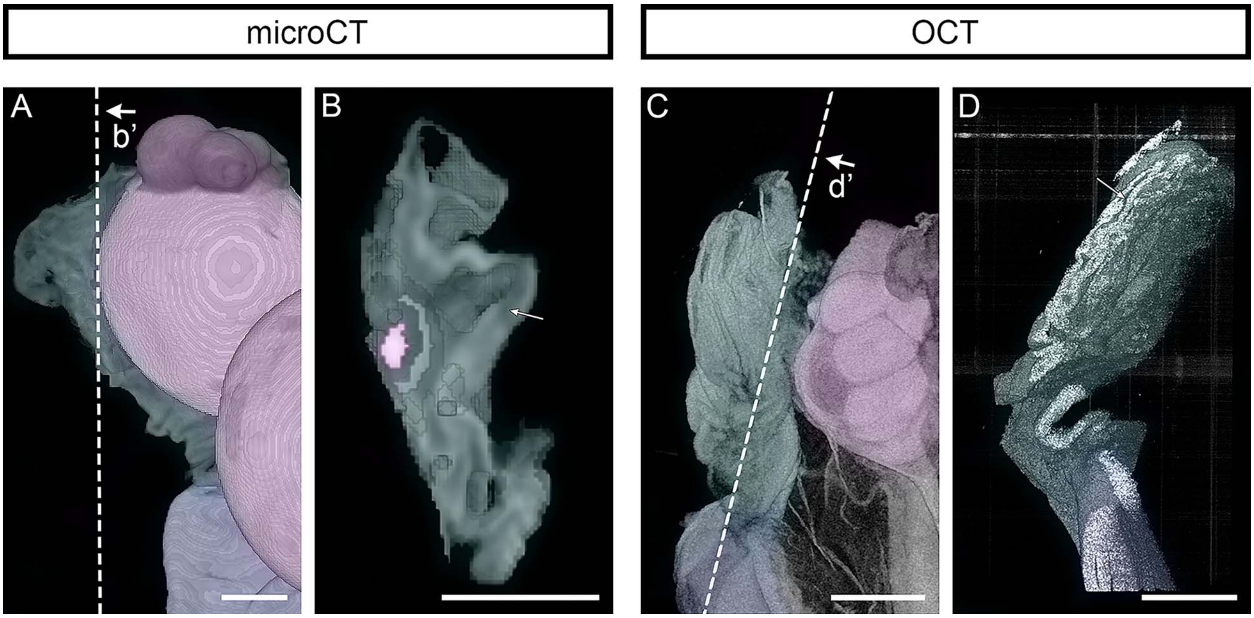

Figure 3. Volumetric imaging of anole lizard infundibulum. (A) Volumetric anterior view of the infundibulum and ovaries imaged with microCT. (B) Volumetric view oriented along the cross-section indicated in panel A as b′, showing the infundibulum structure. (C) Volumetric anterior view of the infundibulum and ovary as imaged with OCT. (D) Volumetric view from the cross-section at the location indicated in panel C as d′, which shows the infundibulum membrane and lumen. Arrows indicate an example location within the lumen. The anatomical structures are color-coded: infundibulum (cyan), ovaries (magenta), and glandular uterus (blue). The scale bars correspond to 1 mm.

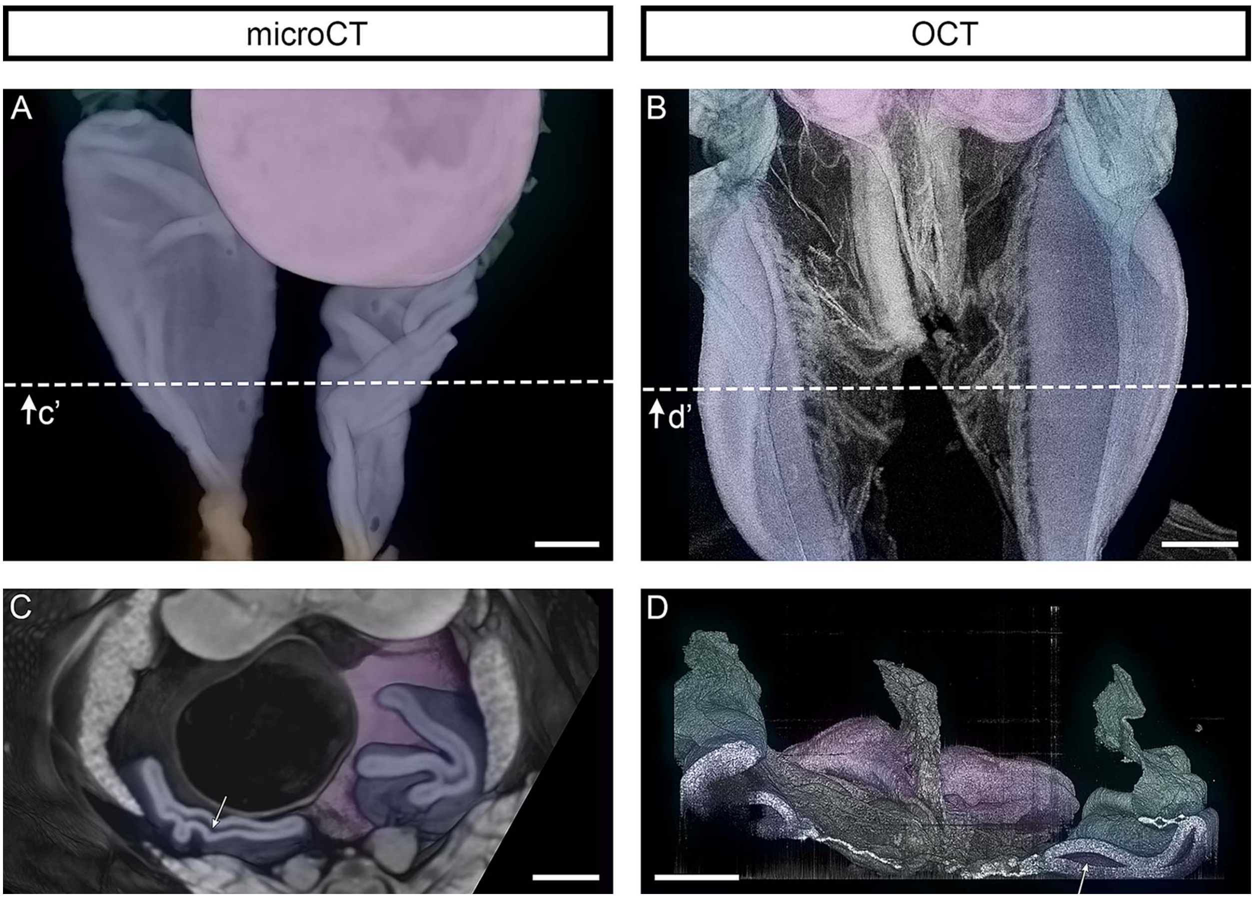

Figure 4. Volumetric imaging of anole lizard glandular uterus. (A) Anterior view of the ovarian follicle and the glandular uterus imaged with microCT. (B) Anterior view of the glandular uterus imaged with OCT. (C) Volumetric view from the cross-section labeled in panel A as c′ showing the microCT scan from the whole body including other organs. (D) Volumetric view of the OCT scan from cross-section labeled in panel B as d′. Arrows indicate an example of the uterine lumen. The anatomical structures are color-coded: infundibulum (cyan), ovaries (magenta), glandular uterus (blue), and nonglandular uterus (orange). The scale bars correspond to 1 mm.

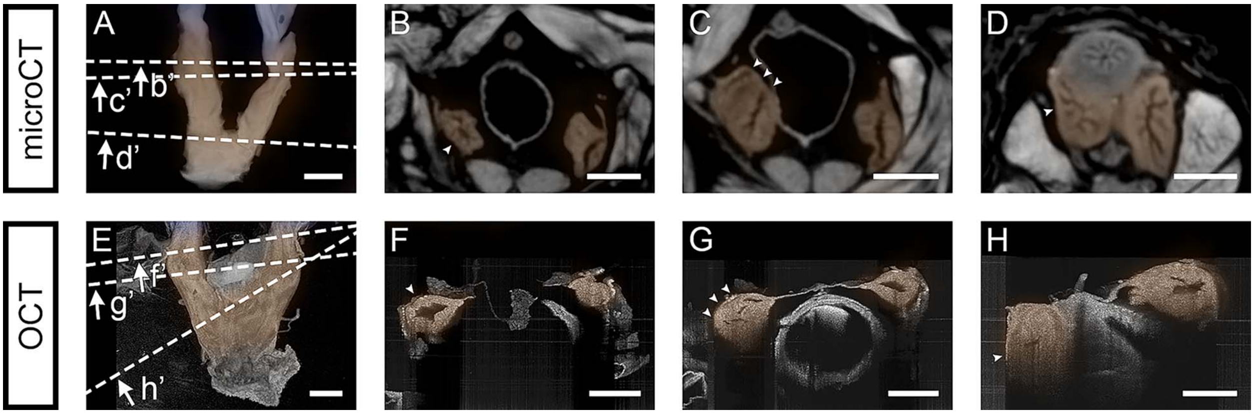

Figure 5. Volumetric imaging of anole lizard nonglandular uterus. (A) Anterior view of the nonglandular uterus imaged with microCT and digitally segmented to show only the reproductive organs. (B-D) View along the cross-sectional corresponding to the locations labeled in panel A as b′-d′ showing the nonglandular uterus (orange) imaged by microCT within the lizard body. (E) Volumetric posterior view of the nonglandular uterus imaged with OCT. (F-H) Volumetric view along the cross-sections indicated in E as f′-h′. Arrows indicate the locations demonstrating defining features of the nonglandular uterus lumen. The reproductive tract is color-coded: glandular uterus (blue) and nonglandular uterus (orange). The scale bars correspond to 1 mm.

Histology of this region shows that the mucosal complexity transitions in a cranio-caudal direction. Cranially, the mucosa, the epithelial lining, is highly folded with a thin muscle layer, while caudally, the mucosa becomes less folded with a more substantial muscle layer. We show, for the first time, that the transitioning mucosa can also be appreciated using multiple volumetric imaging modalities. In the microCT coronal cross-section through the cranial nonglandular uterus, the mucosa appears highly folded, especially in the left tube (Figure 5B). The same features can be appreciated, but are less obvious, in coronal cross section of the OCT dataset (Figure 5F). Furthermore, coronal cross-sections through the mid-region of the nonglandular uterus, called the cervix, show the three distinct luminal segments that are characteristic to this region in both microCT (Figure 5C) and OCT (Figure 5G) datasets. Additionally, in the microCT coronal cross-section through the caudal region of the nonglandular uterus, called the vagina, the lumen has observable characteristics of a stratified, squamous epithelium including a characteristic folded pattern (Figure 5D). The same features can be appreciated, but are less obvious, in coronal cross section of the OCT dataset (Figure 5H).

Quantitative analysis of the reproductive tract wall thickness

To explore the potential of using OCT and microCT imaging data for quantitative structural analysis, we performed measurements of the reproductive tract wall thickness in different regions (Figure 6). The measurements were performed in six animals, three for each modality. While the measurements between different animals are not directly comparable, we find that the measurements across modalities and samples are generally consistent. The animal which was noted to be larger than the rest, Anole 2, had a noticeably thicker reproductive tract across all regions. We found that the wall of the reproductive tract was thinnest in the infundibulum for all animals. The thickest region of the reproductive tract wall was the folded epithelium of the caudal nonglandular uterus. The measurements throughout the glandular uterus were similar across the upper, middle, and lower regions.

Discussion

In this study, we describe for the first time the complete brown anole reproductive tract using two volumetric imaging modalities, contrast enhanced microCT and OCT. Structural reproductive studies among reptiles to date have primarily included histology and electron microscopy, but largely neglected volumetric imaging [2]. In this paper, we show how non-destructive volumetric imaging reveals anatomical context including orientation and relationships between organs of the anole lizard reproductive system. In addition to broad patterns of morphology, both imaging modalities provide the high resolution necessary to capture details such as luminal shape and tissue thickness, which are key anatomical features defining each organ [3]. Performing imaging analysis side-by-side using microCT and OCT allowed us to evaluate the specific advantages and limitations of these two methods for the reptile female reproductive system.

Volumetric imaging techniques represent an exciting avenue of experimentation for developmental and reproductive biologists. Both modalities explored here, microCT and OCT, are non-destructive, volumetric imaging techniques that facilitate high-throughput imaging and preserve three-dimensional information. Such techniques are complementary to traditional histological structural imaging, which identify cell types but are disadvantaged by the destructive nature and loss of three-dimensional data. In this study, we demonstrate that classic histological features of the nonglandular uterus, such as the highly folded luminal mucosa, can also be appreciated in volumetric imaging using microCT and OCT, thus exemplifying their high-resolution capabilities within whole-tissue architecture. We demonstrated that both modalities provide quantitative structural information toward future phenotyping and comparative analyses. The measurements collected here are relatively consistent between modalities and support the architecture of these tissue previously documented with histology [3]. We do observe some variation in measurements. This is likely due to variation in the overall body size of the lizards used in this study as well as due to the variation in tissue processing necessitated by each imaging modality.

The major advantage of microCT is its high resolution and relatively high imaging depth. MicroCT scanning is widely

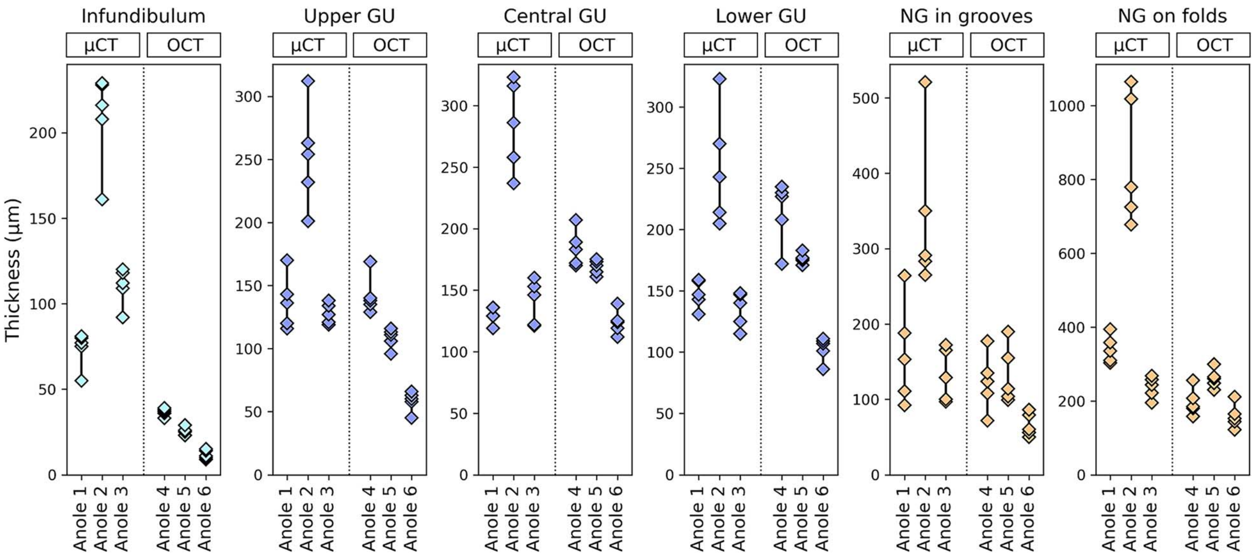

Figure 6. Wall thickness of the reproductive tract measured from two imaging modalities. Five measurements were performed from cross sections of the infundibulum (cyan), glandular uterus (blue), and nonglandular uterus (orange) on each specimen imaged by microCT (n = 3) or OCT (n = 3) as indicated. The first specimen imaged by microCT, Anole 1, and the second imaged by OCT, Anole 5, correspond to the datasets shown in Figures 1–5.

used to study anatomy reaching resolution below 1 micron. Contrast-enhanced microCT, as shown in this study, allows organs of interest to be visualized *in situ* as part of a full-body scan. These advantages of microCT imaging are balanced by specific limitations. While well suited for visualizing organs *in situ*, microCT tissue preparation is relatively labor intensive [26]. Tissue artifacts, such as distortion of morphology, can arise due to tissue fixation and dehydration, as is the case with all histological processes. Similarly, insufficient staining with contrast-enhancing agents can lead to improper stain penetration and incomplete data collection [33]. While staining with improperly buffered iodine can lead to tissue degradation [34], such contrast-enhancing dyes can be removed post-scan by washing, thereby restoring specimens to their pre-stained state [26]. These fixed specimens are stable and suited to long-term storage and future imaging.

The second volumetric imaging modality used in this paper is OCT. This modality has previously never been used to image the reptilian reproductive tract. A major advantage of OCT is that it requires no pre-processing of the tissue and is dye-free, avoiding the tissue artifacts introduced by fixation for microCT, and thereby enabling fresh and *in vivo* tissue analysis. Another advantage of OCT is that the rapid imaging speed allows for dynamic imaging to observe motion in live tissues [35, 36]. However, the limited penetration depth of OCT prevents imaging the reproductive tract through the skin or the other organs in the body cavity, and, in some locations, prevents imaging the full volume of the reproductive organs. In our OCT datasets, the limited depth penetration obscured portions of the glandular and nonglandular uterus.

Both microCT and OCT provide beneficial volumetric information, however, direct comparison between modalities is not feasible as the two technologies inherently rely on different modes of data collection. MicroCT is an x-ray-based technology, and therefore signal is based on tissue density. OCT, on the other hand, is light based, providing data on the optical reflection of near-infrared wavelengths of light. Each imaging modality provides a unique set of data, in that dense tissue is not necessarily more opaque and opaque tissue is not necessarily denser, meaning that signal is not directly comparable. For example, in our comparison, we observe some ovarian follicles in the microCT dataset that have a dense outer rim. In the OCT dataset, we see a fairly uniform reflectivity across each follicle. These differences could reflect either differences in tissue density compared to optical density or variation across stages of follicular maturation.

In this study, we also examined individuals collected from two populations, from Gainesville, FL and Houston, TX. Populations of *A. sagrei* are known to be genetically and morphologically distinct across their range in the southern United States [37]. However, the morphological features discussed here, though understudied in this genus, appear to be largely conserved even between *Anolis* species [9, 13, 16] and appear to be conserved in the specimens used for this study.

Full-body scans, such as presented here, are a well-developed and readily accessible tool for anatomical research as part of an existing repertoire of microCT data. The past two decades have seen the widespread adoption of microCT in non-clinical research and, despite the costs of the instrumentation, there is a growing global network of imaging facilities that employ microCT (https://nocturnetwork.org/resources/ct-lab-world-map/). The beneficial aspects of microCT (non-destructive, scalable, and high-throughput) have resulted in it being used in large-scale efforts to increase the availability of preserved biological specimens via online digital repositories, including The Open Vertebrate Project (oVert, https://www.floridamuseum.ufl.edu/overt/; [38]) and Scan All Fish (https://osf.io/ecmz4/). Open access databases like MorphoSource [39] allow an unprecedented diversity of readily available comparative datasets for CT-based research. These efforts were designed to be taxonomically comprehensive, and so often lack details that can be necessary for small scale analysis including the sex, age, and life history of the specimen; however, the reproductive organs can still be viewed in such scans. We characterize, for the first time, the full reproductive tract from microCT of the female anole lizard and highlight how full-body scans and digital segmentation can be used to study specific organ systems and reproductive anatomy.

While the OCT imaging presented in study was performed on freshly excised samples, there is a potential for dynamic and longitudinal in vivo OCT imaging of reproductive events in anole lizards. Currently, it is not possible to perform *in vivo* OCT imaging non-invasively in adult reptiles due to the optical density of the reptilian scales. However, in vivo OCT imaging could potentially be done through an intravital approach, similar to one previously implemented in mouse reproductive studies. To enable live *in vivo* imaging of the reproductive organs, a surgical procedure has been developed in mice to bypass skin and muscle tissues for imaging by implanting an imaging chamber with a clear aperture overlying the reproductive organs [40]. This technique has been used to study oviductal contractions [40], track oocyte and embryo transport [35], and visualize the trajectories of individual spermatozoa at the site of fertilization [36]. Intravital OCT imaging was also implemented in males for in vivo dynamic investigation of the mouse testis and epididymis [41]. Adapting this intravital imaging technique to adult reptiles would be groundbreaking for the field, where information about the specifics of ovulation, fertilization, and embryo transport is almost completely lacking. Furthermore, OCT has also been applied to capture dynamic ciliary movements within the female reproductive tract *in vivo* [42, 43], which has the potential to provide insight into the role of cilia in egg and embryo transport in mice. Cilia are present throughout the reproductive tract of the brown anole [3, 8], but the ciliary dynamics have not yet been described. OCT live functional analysis is likely feasible in reptiles with adaptation of the methods developed in mice.

In summary, we present volumetric analyses of the female brown anole reproductive tract. This is the first comprehensive review of the anole lizard female reproductive organs as imaged with microCT and the very first time that OCT has been used in this species. We compare these two nondestructive imaging modalities for descriptive analysis of the ovaries, infundibulum, glandular uterus, and nonglandular uterus. The brown anole is one of the most widely studied reptiles in developmental biology research. Recently, genome editing techniques have been developed in this species [44], further contributing to the popularity of the brown anole as a research model. Application of volumetric imaging techniques like microCT and OCT can significantly broaden the questions that can be addressed in this system. This combination of specialized imaging technologies with an under-utilized, non-mammalian model opens further avenues for comparative investigation of reproductive anatomy and addressing fundamental questions in reproductive biology.

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Supplementary material

Supplementary material is available at *BIOLRE* online.

Conflict of Interest: The authors have declared that no conflict of interest exists.

Author contributions

B.K.K, M.A.M, D.M.S, R.R.B, and I.V.L designed this study. B.K.K and M.A.M conducted the experiments and acquired the data. B.K.K drafted the manuscript. B.K.K, M.A.M, D.M.S, I.V.L and R.R.B reviewed and revised the manuscript. R.R.B and I.V.L supervised this study.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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